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Research Article

INFLUENCE OF DUAL INOCULATION OF *RHIZOBIUM* AND MYCORRHIZA ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF *VIGNA RADIATA* (L).

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ABSTRACT

A field experiment was carried out to evaluate the effect of *Rhizobium* and mycorrhiza individually and in combination with *Vigna radiata* L., Experiments were conducted in the field of department of botany, CCS University, Meerut. Results of these experiments revealed that both *Rhizobium* and mycorrhiza have the positive impact on *Vigna radiata* L. growth and yield. However dual inoculation of these bio-fertilizers shows better results in comparison to their individual treatment. Dual inoculation of *Rhizobium* and mycorrhiza increase the growth, yield and biochemical parameters of *Vigna radiata*. So from the present study, we can evaluate that these biofertilizers can be a better replacement of chemical fertilizers.

INTRODUCTION

Pulses constitute an important part of Indian diet and a useful and great source of protein for a huge majority of Indian population. *Vigna radiata* (L.), a protein-rich pulse crop has growing demand in Asia, especially in India because it can provide high-quality protein in Indian diet. Its seeds contain 24.2% protein, 1.3% fat and 60.4% carbohydrates. Calcium and phosphorous are measured as 118 and 340 mg per 100 g of seeds, respectively. It is rich in vitamin-A (Ghildiyal, 1992). There is a need to enhance its productivity to meet the demands of growing population.

The greatest challenge of the 21st century is to produce the basic needs of food and fuel for human and animal consumption from the limited available land without degrading and harming soil and at the minimum amount of agriculture inputs. The accessibility of land for agriculture is contracting each day as it is progressively used for non-farming purposes while physical and synthetic properties of soils are everyday changing disgracefully which influence generation and appropriateness of the soil to various many products (Patra, 2009). Under this situation, one of the important strategies to increase agriculture production as well as to improve and sustain soil properties is the development of high use efficiency of all sources of nutrients available.

Legume crops can be grown in low water and low nutrient soil due to their capability to form symbiotic relationship with soil microorganisms (Kawaguchi *et al*., 2010). Phosphorus and nitrogen constitute the most limited nutriment for vegetative growth. In order to assess the capacity of the plant to obtained and solublize nutrients, arbuscular mycorrhizal fungi and rhizobia are two of the most important plant symbionts. They play a key role in natural ecosystems and influence plant productivity, plant nutrition and improved inhibition of fungal plant pathogens (Demir and Akkopru, 2007). Mycorrhiza benefits the host through mobilization of phosphorus from non-labile sources, whereas *Rhizobium* have main role in biological nitrogen fixation (Scheublin and Vander Heijden, 2006).

Rhizobium belongs to a group of bacteria, in the family of Rhizobiaceae and is a symbiotic nitrogen fixer in association with legume plants only. They are the most efficient bio-fertilizers in terms of per quantity of nitrogen fixed. The bacteria infect the roots of the legumes and form root nodules within which they fix nitrogen (reduce atmospheric nitrogen to ammonia). Leguminous plants utilized this nitrogen to produce nitrogen-containing compounds such as proteins, vitamins, etc. (Mortimer *et al*., 2008).

The arbuscular mycorrhizal fungi produce highly complex and branched hyphal structure within the plant cell. This hyphal structure within the plant cells increases the surface area of

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plant cells for the absorption of nutrients from soils. This infection creates an absorptive structure with a very high surface area of transfer for nutrients between the plant and the fungus. Acid and alkaline phosphatases (APA and ALP) were secreted into the rhizosphere by mycorrhiza hyphae. It was established that APA activity increases in roots growing under P stress (Woolhouse, 1975). Therefore, the regulation of these enzymes is critical to a plant's survival in soils with limited Phosphorus resources (Duff *et al.,* 1991). There is extensive evidence for a decrease in the number of arbuscules under high external P (Bethlenfalvay *et al*., 1990, Smith and Smith, 1996). It will be better if the maximum supply of P and N for production can be biologically obtained with mycorrhizal fungi and *Rhizobium*, respectively. But fewer attempts has so far been made on the co-inoculation of *Rhizobium* and mycorrhizal fungi both to improve the yield of mungbean. Therefore, it is essential to determine the influence of AM with *Rhizobium* based on the symbiotic nitrogen fixation and N and P nutrition of mungbean.

The major objectives of the present study are to address the interactive effects of AM fungi and *Rhizobium* on *Vigna radiata* (L.) growth and nutrient uptake.

MATERIAL AND METHODS

The present experimental work was conducted to evaluate the 'effect of dual inoculation of *Rhizobium* and Mycorrhiza on the morpho-physiological properties of *Vigna radiata'.*

Geographical situation

The University is situated at the distance of about 10km from Meerut city railway station. The total geographical area of Meerut district is 2564km^2. The region falls under western plain zone of Uttar Pradesh, sub locale of upper Gangetic plain. The research work was conducted at experimental field of Department of Botany, Chaudhary Charan Singh University, Meerut, during Kharif season (April to June 2017).

Material used

1. Pure seeds of *Vigna radiata* (L) were acquired from Indian Agricultural Research Institute, New Delhi.
2. *Bradyrhizobium* and mycorrhiza was also procured from Indian Agricultural Research Institute, New Delhi.

Experimental design

Experimental details of present work are as follows;-

1. Total no. of block – 6
2. Control - 1
3. Total no. of treated plots – 5
4. Plot size – 1X1 meter

The experiment was designed in six plots of equal size, one plot for the control and five plots for the treatment. Fifty healthy seeds of *Vigna radiata* (L.) were sown in each plot. Six plots designed for present work are as follows;-

1. Control
2. *Rhizobium* treatment
3. Mycorrhiza(20gm) treatment
4. Mycorrhiza(15 gm) treatment+*Rhizobium*
5. Mycorrhiza(20 gm)treatment+*Rhizobium*
6. Mycorrhiza(25 gm)treatment+*Rhizobium*

Mycorrhiza application

2 kg soil taken from each block and different concentrations of VAM (Vesicular arbuscular Mycorrhiza) 15 gm, 20 gm, and 25 gm were mixed in this soil evenly and then it is mixed in last three blocks uniformly, before sowing of seeds.

Rhizobium application

For seed inoculation with *Rhizobium,* we prepared the *Rhizobium* slurry. For preparation of *Rhizobium* slurry, 2.5 gm of *Rhizobium* was added with 50 gm of sugar (used as adherent) stirred in 500 ml of water. After mixing them properly we boil the mixture until the slurry became sticky. Seeds are mixed in this slurry for 30 minutes or until the *Rhizobium* did not adhere to the seeds uniformly.

Parameters

Seed germination

The seed germination % was calculated after counting the difference between non-germinated seeds (remaining inside, non emergent) and germinated (coming out of the soil). Germination percentage was calculated by using following formula-

Germination percentage = Seeds germinated/total seeds x 100

Nodulation parameters

Five plants were uprooted from each plot after 30 days of sowing (DAS) and were observed for the extent of nodulation parameters such as number, weight (fresh & dry) and volume of nodules.

Growth parameters

Growth parameters such as plant height (Root and shoot) and Plant weight (fresh and dry) were observed to study the effect of *Rhizobium* and Mycorrhiza on plants morphological characters. Five plants were removed from each plot and then measured for further experiment.

Yield parameters

Plants (5 plants from each plot) of six plots were observed for different yield parameter such as numbers of pods per plant and numbers of seeds per pod**.**

BIOCHEMICAL PARAMETERS

Estimation of total proline

Total proline contents of nodules were estimated by the following protocol given by Bates *et al*., (1973).

Determination of Protein

The protein was estimated by Bradford method. To determine the protein contents in the nodules, the plants were uprooted carefully from the soil and washed thoroughly to remove soil particles. Protein concentration of the samples were determined by using standard curve and calculated as mg/gm fresh weight (Bradford. 1976).

The following formula was used for the measurement of protein content

$$\frac{OD \text{ x factor x dilution (if any) x } 1000}{100 \text{ x total volume}}$$

Leghaemoglobin content analysis

For leghaemoglobin analysis, the plants were uprooted carefully from the soil, washed thoroughly to remove soil particles and nodules were removed carefully. Leghaemoglobin quantities of the nodules were measured spectrophotometrically as hemochromogen according to the method of Bergersen (1980). Then calculated the leghaemoglobin content by using the following formula

$$\text{Lb Protein} = \frac{\text{LB/g fresh weight of nodule X 100}}{\text{Protein/g/fresh weight of nodules}}$$

Estimation of Chlorophyll content

Chlorophyll content was estimated by using Arnon's method. For calculation the following formula is used-

$$\text{Chl. a (mg/g f wt)} = \frac{12.7(A663) - 2.69(645)X\ V}{1000X\ W}$$

$$\text{Chl. b (mg/g f wt)} = \frac{22.9(A645) - 4.89(A663)xV}{1000xW}$$

$$\text{Total Chl. (mg/g f wt} = \frac{20.2(A645) - 8.02(A663)x\ V}{1000xW}$$

Where,
V= Final volume of chlorophyll extract
A= Absorbance at specific wavelength
W= Fresh weight of tissue extract

RESULT AND DISCUSSION

Germination percentage

Seed germination values in the mycorrhiza treated seeds were reported higher than those in control. Maximum germination was recorded in treatment 6 (25 gm mycorhizza with *Rhizobium*). However, there are gradual increases in germination percentage of all the treated plots over control where neither *Rhizobium* nor mycorrhiza applied. Both microorganisms have positive impacts on seed metabolism. Through biological processes these symbiotic microorganisms can change prime nutrients from an unavailable form to an available form, extend the root system and improve seed germination (Chen, 2006). The fixation capability of *Rhizobium* is a factor in sustainable agriculture point of view. Nitrogen is fixed and then enriches the soil in terms of nitrogen and thus soil properties are subsequently improved and also improve the germination capacity of legumes.

Plant growth

The plants inoculated either with *Rhizobium* or mycorrhiza and dual inoculation (mycorrhiza with *Rhizobium*) significantly increased the shoot length and root length of plants, but maximum plant height (77cm) was observed when dual inoculation of mycorrhiza (25 gm) and *Rhizobium* was given to plants. Minimum plant height was observed in control (64.6 cm). The result showed the synergetic effect of co-inoculation of *Rhizobium* with mycorrhiza in improving growth of *Vigna radiata* when compared to single inoculation of both individually. Plants required nitrogen and phosphorus in a large amount and generally, these macroelements are depleted in the soil or available in the insoluble form which plants cannot absorb. Mycorrhiza solubilizes the phosphorus and make it available to the plants. On the other hand *Rhizobium* fixes nitrogen with legumes symbiotically and make atmospheric nitrogen available to the plants. Dual inoculation of these microorganisms provides optimum nutrients to the plants for growth. Similar results were observed by Tajini *et al.* (2011) and Havugimana *et al.* (2015).

Plant biomass

Minimum plant dry weight (root+ shoot) was observed in control. Mycorrhiza and *Rhizobium* both have positive impacts on plant growth so plant biomass gradually increased in all treatments when *Rhizobium* and mycorrhiza were given individually or in combination. However, maximum results were obtained when co-inoculation of *Rhizobium* and mycorrhiza were given. This could be due to the improved absorption of inorganic nutrients, especially of phosphorus and greater rates of photosynthesis in inoculated plants. The extensive mycorrhizal hyphal network could have enabled legumes to acquire phosphorus from distances beyond the nutrient depletion zone of the roots as well as to solubilize phosphorus from unavailable sources (Marschner, 1992). Mycorrhizal plants could also have obtained phosphorus from normally unavailable sources of both inorganic and organic forms (Koide and Kabir, 2000; Feng *et al*., 2003). The enhanced phosphorus could have been utilized in the nodules as an energy source in biological nitrogen fixation where atmospheric nitrogen is reduced to ammonia (NH3) which is taken up by the plant and assimilated into amino acids, leading to increased plant biomass (Hogberg, 1986).

Nodulation

Minimum nodules number (12) was observed in un-inoculated control plants and in the plants inoculated only with the mycorrhiza (13). Plants treated with dual inoculation of *Rhizobium* + mycorrhiza nodulated well. The number, size, dry weight and volume of nodules in plants inoculated with mycorrhiza and *Rhizobium* were significantly greater than those of plants inoculated with *Rhizobium* and mycorrhiza individually. The maximum values were observed in treatment 6 (*Rhizobium*+ 25 gm mycorrhiza). Dual inoculation improved the nodulation capacity of *Vigna radiata* plants. Mycorrhiza infection of the legumes root has positive impact on *Rhizobium* population. It is known to stimulate both nodulation and nitrogen fixation especially in soils low in available phosphorus. Olsen and Habte (1995) came to the same conclusion when investigating the effect of mycorrhizal inoculation on nodulation and N accumulation in *Cajanus cajan*. It is also thought that the plant–*Rhizobium* symbiosis benefits from the presence of AM fungi because the mycorrhiza ameliorate not only phosphorus deficiency but also any other nutrient deficiencies that might be limiting to *Rhizobium* (Smith, 2002). Increased mineral nutrient levels in the plants would not only benefit *Rhizobium* directly but would also lead to increased photosynthesis, making a greater proportion of photosynthates available to the *Rhizobium* nodules (Mortimer *et al*., 2008). According to Nautiyal *et al*. (2010) the dual inoculation of *Cicer arietinum* L. with

Rhizobium and AMF significantly enhanced the number of nodules and the dry weight per plant. Such results do also have harmony with Molla *et al*. (2014).

Yield parameters

Yield parameters (no. of pods per plant and no. of seeds per pod) were reported higher in treatment 6 when co-inoculation of *Rhizobium* with mycorrhiza (25gm) was applied than those in control. Minimum no. of seeds per pod and pods per plant was recorded in control. Co-inoculation of *Rhizobium* with mycorrhiza has positive impacts on yield parameters. This may have happened due to the availability of nutrients to the plants in optimum amount. The rate of nutrients uptake may be modulated by the presence or absence of AM Fungi or *Rhizobium* inoculation in leguminous crops. Hyphae of AM fungi extends into root zones even beyond the plant rhizosphere and take up nutrients as well as transport them into the rooting zone. AMF contributed substantially to Cu, Zn and Cd uptake in the bean. AM Fungi and rhizoidal combination suppressed the tendency for the acquisition of seed Al, Cu and Fe. Phosphorus and nitrogen have major role in plant metabolism. These are the major elements of proteins and nucleic acids. Nitrogen has positive impact on plants protein and carbohydrates. It is observed from different studies that when the amount of nitrogen increases, more protein is produced because of nitrogen as the building block of amino acid. So mycorrhiza and *Rhizobium* have positive impacts on plant yield characters.

Chlorophyll content

Results revealed that maximum chlorophyll content was observed in treatment 6 (*Rhizobium*+ 25 gm mycorrhiza) and minimum chlorophyll contentwas observed in control. Plants inoculated with AM fungi, either alone or in combination with *Rhizobium*, brought about significant changes in chlorophyll *a*, *b* and its total content. Maximum total chlorophyll content was found in dual inoculated plants followed by individual inoculation of AM fungi and *Rhizobium*. A considerable increase in chlorophyll content of AM fungi and *Rhizobium* inoculated tissue of mungbean is in agreement with results reported by Hayman (1983). This increase may be due to an increase in stomatal-conductance, photosynthesis, transpiration and enhanced plant growth (Rajasekaran *et al*., 2006) or due to the presence of large and more numerous bundle sheath chloroplasts in the inoculated leaves (Krishna and Bagyaraj, 1984). These results also have similarity with the results obtained by Arumugam *et al*.(2010).

Leghaemoglobin content

Results obtained from the present study reveal that the maximum leghaemoglobin content was observed in treatment 6 and minimum in control. Leghaemoglobin occurs in the root nodules of leguminous plants, where it facilitates the diffusion of oxygen to symbiotic bacteria in order to promote nitrogen fixation. Leghaemoglobin is known to the direct influence on the nodulation efficiency of leguminous plants because it provides the suitable environment to *Rhizobium* for nitrogen fixation. Dual inoculation has positive impacts on leghaemoglobin content. It may be due to the function of AM in nutrient uptake and positive impacts on legume-*Rhizobium* symbiosis. AM fungi can improve phosphorus uptake by the plant which in turn would result in more energy available for nitrogen fixation by *Rhizobium*. Dual inoculation showed synergistic effects on nodulation and N2 fixation in P-deficit soils. The uptake of other essential micronutrients from the soil by the AM fungal hyphae might also play a role in general plant growth improvement as well as in more indirect effects upon the N2-fixing system.

Protein content

Protein content was found maximum when *Rhizobium* with mycorrhiza (25 gm) was applied. The minimum amount of protein was observed in control. All the treatments of *Rhizobium* and mycorrhiza have positive impact on protein content either as individually or in combination. The results clearly indicate that inoculation of the plants with both *Rhizobium* and mycorrhiza increased the nitrogen and phosphorus contents of mungbean. There was an overall increase in nitrogen and phosphorus contents in the treated plants as compared to control. However, maximum nitrogen and phosphorus contents were recorded in the plants dually inoculated with mycorrhiza and *Rhizobium*. Inoculation of legumes with *Rhizobium* increases the nodulation of legumes causing more nitrogen fixation and making it available for the plants. The increase in protein content was due to the fact that phosphorus is an important structural component of DNA and RNA. The phosphate group in nucleic acids bridges the RNA or DNA. DNA is the carrier of genetic information and RNAs function in protein synthesis. Similarly nitrogen is the building block of amino acids. So there is a positive relationship between the nitrogen and phosphorus availability and protein content. Such results have also been recorded earlier by Bhattacharjee *et al.* (2012).

Proline Content

Proline is a major amino acid, which is essential for primary metabolism (Tan *et al*., 2011). Higher plants which are tolerant to water deficits and salinity stress usually accumulate higher levels of proline to cope with the harsh environments (Dinaker *et al*., 2009). Maximum proline content was measured in control while minimum amount of proline was observed in treatment-6 (25 gm mycorrhiza + *Rhizobium*) as compared to all other treated plants. It was obsereved that the application of high amount of mycorrhiza with *Rhizobium* were responsilble for the decrease in proline contents of plants.

Graphs

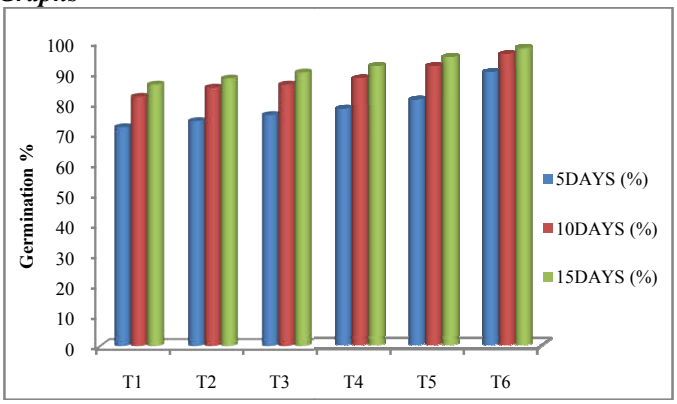

Figure 1 Seed germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.(T1-Control,T2- *Rhizobium* treatment,T3-Mycorrhiza(20gm),T3-Mycorrhiza(15gm)+*Rhizobium,*T4-Mycorrhiza(20gm)treatment+*Rhizobium,*T6-Mycorrhiza(25gm)treatment+*Rhizobium.*

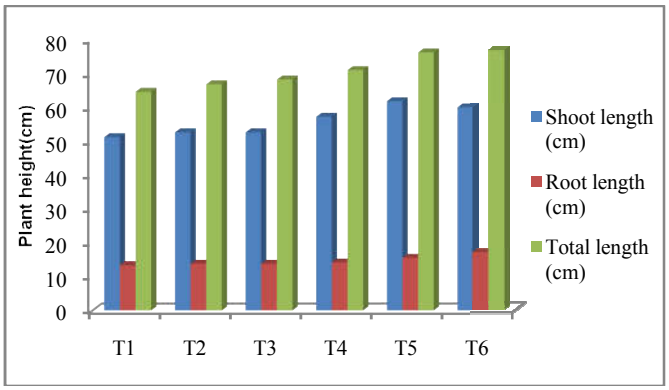

Figure 2 Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

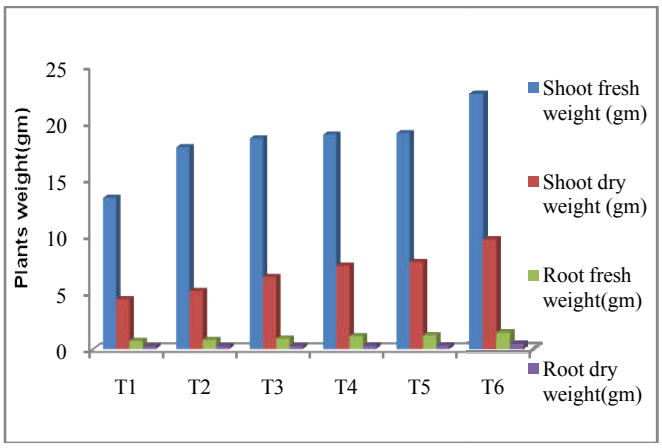

Figure 3 Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

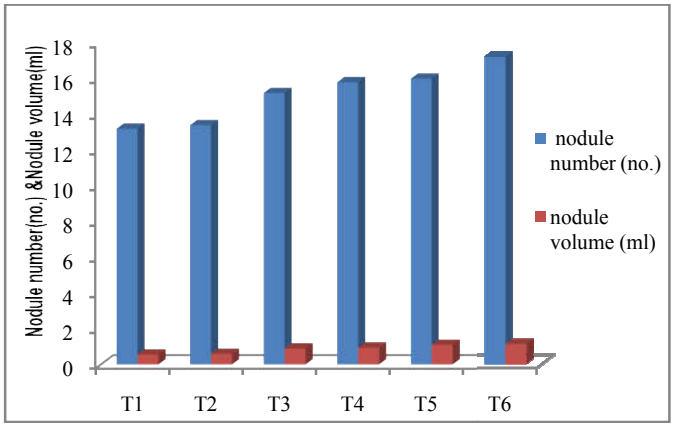

Figure 4 Nodule number and nodule volume of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

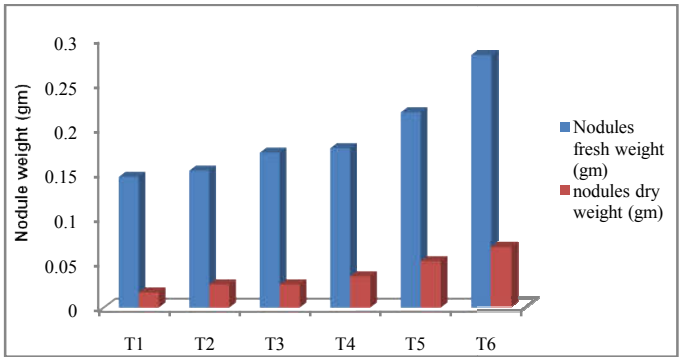

Figure 5 Nodule fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

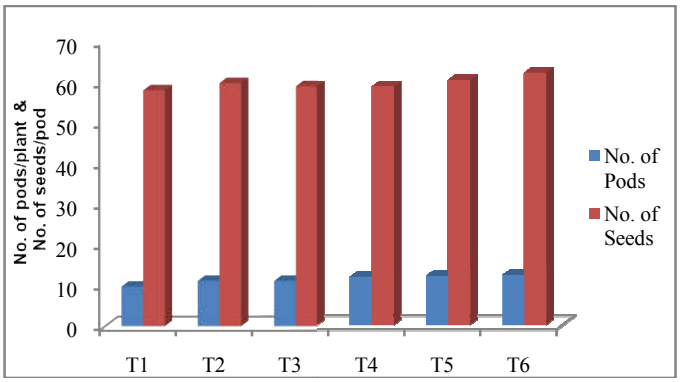

Figure 6 Yield parameters (number of pods and seeds) of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

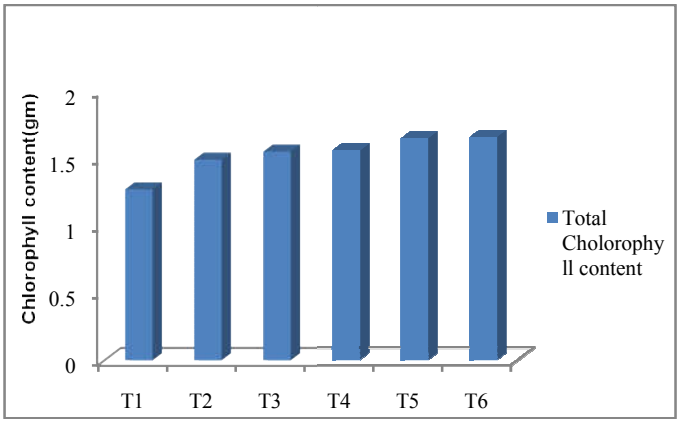

Figure 7 Chlorophyll content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

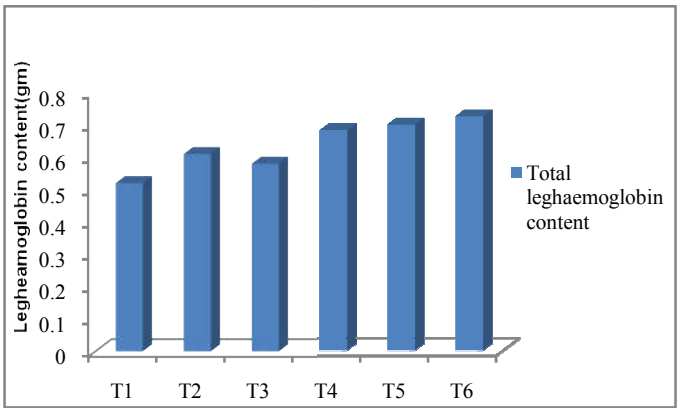

Figure 8 Leghaemoglobin content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

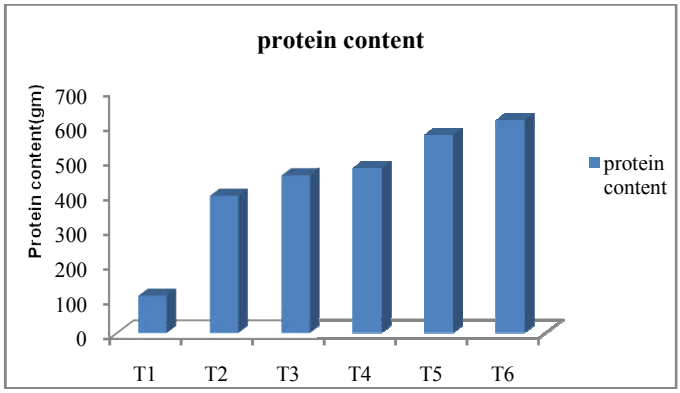

Figure 9 Protein content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

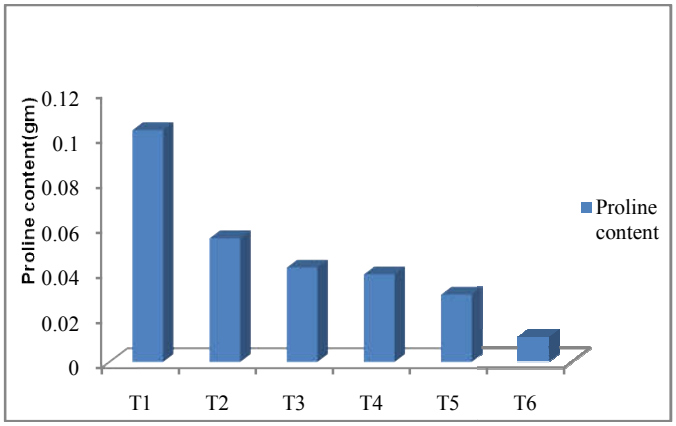

Figure 10 Proline content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of mycorrhiza.

CONCLUSION

It can be concluded from the present study that *Rhizobium* and mycorrhiza both have the positive impact on *Vigna radiata* growth and its biochemical parameters either these are given to plants individually or in combination. The present study revealed that that the dual inoculation of Rhizobium and mycorrhiza give better results in comparison to their individual treatments. In present time we need more food production to fulfill the requirement of a huge population of India as well as we have responsibilities to save the environment from the harmful effects of chemical fertilizers which we used drastically to increase the crop yield. So we need a replacement of these harmful chemical fertilizers which can improve the plant yield as well as should be eco-friendly, these bio-fertilizers can be a better replacement of chemical fertilizers.

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